The protective ability of glutathione may therefore be due to its reactions with the oxidizing agents produced by irradiation. Such reactions would decrease the amount of reactive ionization products available for reactions with the chromosomes and result in fewer breakages.

Apparently, a maximum protective effect was obtained at the two highest concentrations, but it is not known whether the maximum occurred because the glutathione reacted with all the oxidizing agents produced by the radiation or whether the maximum possible absorption of glutathione in the cell nuclei was obtained at the second highest concentration used (3×10^{-4} *M*). In the absence of oxygen the aberration frequency was reduced to one third in some experiments (*4*). This may indicate that the optimal effect of oxidizable chemicals on chromosome aberrations is not obtained with glutathione, which gave a reduction of about one half in these experiments. If these arguments are correct, however, the results indicate the importance of the indirect effect of the oxidizing reactants produced in water by radiation in the process of chromosome breakage. The data presented are preliminary, and further work is being conducted on various aspects of the problem.

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Absorption Spectra and Isomerization of the Chlorophylls

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In the search for clues to the mechanism of photosynthesis, several isomerization reactions and numerous variations of the absorption spectra of the chlorophylls have been observed. These isomerizations and spectral changes take place in killed plant tissue and in extracts of plants. They have not been observed during the photosynthetic utilization of sunlight by living plants (*1, 2*).

The wavelength of the absorption maxima of the chlorophylls depends upon the physical state or condition of these pigments. In living organisms, in many colloidal suspensions, and in the solid state, chlorophyll *a*, the principal photosynthetic pigment, exhibits a spectral absorption maximum near 680 mμ (*2*). But in solution in organic solvents, this chlorophyll exhibits a spectral absorption maximum at shorter wavelengths (about 660–673 mμ, depending upon the solvent). The similarity between the spectral properties of chlorophyll *a* in living plants and in colloidal dispersions indicates that this green pigment may occur naturally in colloidal form (*2*). In colloidal suspensions prepared by grinding plant tissues, however, the chlorophyll is always associated with carotenoid pigments and with various colorless substances such as proteins and fats. Photosynthetic activity has been observed only in living plants in which the chlorophyll occurs in this special labile association (*2, 3*).

Reversible isomerization reactions of the chlorophylls take place spontaneously in solutions of the pigments (*4*). These isomerizations are not accompanied by significant spectral changes. Chlorophyll *a* yields the similar chlorophyll *a'*. Chlorophyll *b*, the minor green pigment of green algae and of higher plants, also yields a similar interconvertible isomer, chlorophyll *b'*. These isomeric chlorophylls are usually detected and isolated by chromatographic adsorption in columns of powdered sugar.

Spectral changes of the chlorophylls occur when the solutions in hydrocarbon solvents are cooled (*5*). With decreasing temperature, the maxima are shifted gradually to longer wavelengths, from about 663 mμ at 293° K to 675 mμ at 75° K for chlorophyll *a*, and from about 643 mμ at 293° K to 660 mμ at 75° K for chlorophyll *b*. These shifts of the spectral absorption maxima have been ascribed to a reversible isomerization of the chlorophylls, the nature of the isomers being unknown.

Pronounced shifts of the spectral absorption maxima of the chlorophylls dissolved in petroleum ether plus methanol have now been observed when the alcohol is removed from the solutions. These spectral shifts, which are influenced greatly by the presence of colorless impurities and by the solvent itself, may be greater than those observed when the usual preparations of chlorophyll are dispersed in water, or when solutions of the chlorophylls in hydrocarbons are cooled. The shifts produced by the removal of alcohol from petroleum ether solutions of the highly purified chlorophylls are due to variation of the physical state of the pigments, not to the formation of isomeric substances.

For observation of these spectral shifts, the chlorophylls and their isomers were extracted from heated leaves and were separated by chromatographic adsorption with powdered sugar as adsorbent and with freshly washed and distilled (bp, 35°–40°) petroleum ether plus 0.5% propanol as solvent. The chlorophylls, separated in the column as four green zones, were eluted from the respective portions of the sugar with the low-boiling petroleum ether containing about

5% methanol. The methanol was then removed from the clear, green extracts by contact with water. In a few minutes, the absorption maxima of the solutions shifted to longer wavelengths, chlorophyll *a* from 663 to about 710 mμ, chlorophyll *b* from 643 to 690 mμ. Chlorophyll *a'* behaved like chlorophyll *a*, chlorophyll *b'* like chlorophyll *b*.

For reproducibility of these spectral shifts, low-boiling petroleum ether should be employed instead of higher-boiling fractions (50°–60°). The chromatogram should be developed extensively in order to remove colorless, fatty substances. Only the more concentrated regions of the chlorophyll zones in the chromatographic columns should be utilized, thereby reducing the amount of colorless contaminants. With extracts of some plants, readsorption of the chlorophylls was necessary. The removal of the methanol and residual propanol from the petroleum ether solutions must be complete. The entire preparation, including extraction, separation, and recovery, should be carried out in a short time (about 1 hr).

Chlorophylls in the alcohol-free petroleum ether were present in insoluble, colloidal, or microcrystalline form. These suspensions were turbid and were very weakly fluorescent. When the suspensions were centrifuged or were filtered through paper or shallow layers of powdered sugar, most of the pigments were removed, leaving very light green solutions with absorption maxima at shorter wavelengths. Chlorophylls *b* and *b'* yielded such dilute solutions that a depth of 10 cm was required in order to reveal the spectral absorption maximum at 645 mμ. Resuspension of the centrifuged pigments in fresh petroleum ether provided turbid mixtures with reappearance of the spectral absorption maxima at the longer wavelengths. Agitation of these centrifuged chlorophyll preparations with water in a stream of nitrogen also provided suspensions with absorption maxima at the longer wavelengths. The addition of traces of fats, sterols, higher aliphatic alcohols, and paraffin to the petroleum ether plus methanol solutions, followed by removal of the methanol with water, yielded solutions or suspensions with spectral absorption maxima ranging from those of the true solutions of the chlorophylls to those of the suspensions of the purified pigments.

The purified chlorophylls precipitated from petroleum ether were not altered chemically, and they were not isomerized. These preparations redissolved rapidly in petroleum ether in the presence of a little methanol or acetone. The resultant solutions contained the original unaltered chlorophyll, as shown by spectral absorption properties and by the formation of a single zone in the chromatographic column. They also formed a single zone when adsorbed with some of the unprecipitated chlorophyll. The precipitation, dissolution, and adsorption were repeated several times with a single chlorophyll preparation without evidence of alteration.

In summary, the absorption maxima of the chlorophylls purified by chromatographic adsorption are shifted to much longer wavelengths when these pigments are precipitated from solution in petroleum ether. These spectral shifts, which are analogous to those observed by cooling solutions of the pigments in hydrocarbon solvents, are due to a change in the physical state of the pigments, not to isomerization. The differences between the spectral properties of chlorophyll *a* in plants and in various pigment preparations support the view that this photosynthetic substance occurs naturally in a unique combination or association with other constituents of the chloroplasts.

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Equilibria between Species of Chlorophylls in Solution[1]

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In order to clarify the interpretation in our previous communication (*Science,* **114**, 275 [1951]), to which H. H. Strain refers in the preceding article, we shall discuss some aspects of our work in more detail and present more recent results in confirmation of our point of view. At the same time, we shall show that the behavior of the colloidal solutions of chlorophyll studied by Dr. Strain cannot account for the changes we have found in the spectra of chlorophyll in solution.

The existence of isomers was not inferred from shifts in wavelength of any of the spectral peaks with temperature, since all the peaks of each species moved toward longer wavelengths as the temperature was lowered. Rather, the presence of two isomers in each of the chlorophylls in the ether-hydrocarbon solvent was implied by the coexistence of two similar systems of peaks, the relative intensities of which varied reciprocally upon change of temperature. Actually, the corresponding peaks were clearly resolved in the blue region of the spectra of chlorophylls *a*, *b*, and *b'*, and on the short wavelength side of the red peak of chlorophyll *a*. However, the main red peak in each of the three chlorophylls appeared single. It was surmised that the main red peaks of the pairs of isomers were superimposed on each other and that they shifted with temperature to about the same degree. Indeed, there were indications that the superpositions were not exact.

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